REMARKS

Claims 1-24 are pending in the present application. Claims 15-24, have been withdrawn as being directed to non-elected subject matter. Claims 1, 3, 7, 10 and 13 have been amended. Support for the amendments is found throughout the specification. No new matter has been added by virtue of these amendments and their entry is respectfully requested.

Amendment and cancellation of the claims are not to be construed as an acquiescence to any of the rejections/objections set forth in the instant Office Action, and were done solely to expedite prosecution of the application. Applicants reserve the right to pursue the claims as originally filed, or substantially similar claims, in this or one or more continuation or divisional patent applications.

Claim Rejections Under 35 U.S.C. § 112, first paragraph.

Claims 1-14 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

Applicants respectfully traverse.

Applicants discuss and present experimental data on the functional activities of the purified nucleic acids encoding a myofibrillogenesis inducing RNA (MIR). Applicants describe the parameters for assessing induction of cardiac muscle phenotype in a cell. These include: induction of rhythmic contraction in a treated cell in culture; onset of beating of the heart *in vivo*; use of morphological and immunohistochemistry, such as detection of tropomyosin; MIR-protein binding experiments such as Northwestern blots, antibody probes; Northern blotting, Western blotting, PCR, gel-shift assays and the like. See for example, page 14, lines 10-28 through to page 16, lines 1-19. Any of these methods can be used to assess whether a mutant, variant, homolog as disclosed on page 12, lines 20-28 through to page 14, lines 1-8. Identification of those molecules to bind to an MIR-binding protein is shown in figure 3 wherein the mutant MIR contains a G to C point mutation in position 93, encoded by SEQ ID NO.: 4. (See, page 12, lines 6-19).

Assays to determine the functional activities of the MIR encoding nucleic acids are described in detail in the Examples section. Example 1, page 21, lines 5-27 through to page 22, lines 1-9 describe an organ culture system for heart morphogenesis, including a bioassay to

determine rescue of cardiac lethal mutant hearts. Example 5, page 24, lines 5-35 describes the functional parameters of MIR encoding nucleic acids, such as measuring tropomyosin and heart beating. See Table 1 for results.

Example 2 on page 22, lines 10-27 through to page 23, lines 1-2, describes the effects of RNase on the MIR-encoding nucleic acids which shows that the enzyme destroys the activity of these nucleic acids. See Table 1 for results and functional activities tested. Example 6, further describes the rescue of myofibrillogenesis in mutant hearts by sheep heart RNA. Results are shown in figure 1 indicating that myofibrils with thick and thin filaments were visible in rescued cells." Page 25, line 27.

Example 7 describes various methods used to analyze the RNA mediating mutant heart rescue. (See page 26, lines 3-28 through to page 27, lines 1-12). Included in this example is a method used for determining the secondary structures of both normal and mutant MIR RNA:

RNA secondary structures of normal and mutant MIR RNA were predicted by Genebee software, an internet-based server for analyzing biopolymer structures (Brodsky et al., 1995). The method applied in this software for RNA secondary structure prediction uses phylogenetic considerations for energy optimization. Because no MIR homologous sequences have been identified, the RNA secondary structures generated by the software and chosen for comparison were based solely on the lowest energy (free enthalpy of the structure).

Example 12 describes the determination of secondary structure of a mutant MIR. See page 30, lines 15-27 through to page 31, lines 1-2. The results as well as the changes in secondary structure by the mutant are shown in figure 5. The disclosed mutant significantly altered the secondary structure as compared to the normal MIR molecule to alter the functional activity of the mutant. Coupled to the secondary structural analyses are the results from the disclosed methods of determining the functional activities of the MIR encoding nucleic acids. For example, Example 13 describes how to determine the ability of a mutant to bind protein, using a mutant with an altered secondary structure. The results of the experiments are shown in figures 6A and 6B. Thus, the sequence, structure and function of the MIR-encoding nucleic acids are easily correlated based on the applicants disclosure.

Applicants therefore, have described in detail the functional activities of the MIR encoding nucleic acids, the identity of the structural features based on the nucleic acid sequences,

including mutant MIR-encoding nucleic acid sequences. Based on the above and the disclosure in the instant application, Applicants submit they were in possession of the invention as claimed. Applicants respectfully request reconsideration and withdrawal of the rejection.

Claim Rejections Under 35 U.S.C. § 112, second paragraph.

Claims 1-3, 7, 10, 14 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as his invention.

Applicants respectfully traverse.

The Examiner alleges on page 4 that the functional activity of a native MIR molecule, is unclear.

The functional activities of the MIR-encoding molecules and methods of determining whether an MIR-encoding nucleic acid has any functional activities has been discussed above. Briefly, the functional activities of the MIR-encoding nucleic acids, disclosed in the instant invention, include: binding to MIR proteins, induction of rhythmic contraction, myofibrillar induction, induction of differentiation of a cell into cardiac muscle phenotype.

The functional activities associated with the MIR encoding nucleic acids are summarized by applicants on page 9, lines 1-15:

> Myofibrillogenesis inducing RNA (MIR) is an RNA molecule expressed in embryonic endoderm, with the ability to induce formation of myofibrils in differentiating cardiomyocytes of normal, but not mutant individuals, in an animal model of heart development.
>
> In studies disclosed herein, the full-length nucleotide sequence of MIR is disclosed. It is further shown that MIR extracted from adult mammalian (sheep) heart has the ability to promote ("rescue") heart cell differentiation in mutant salamanders, enabling these cells to exhibit normal rhythmic contractions, tropomyosin distribution, and myofibril formation. Detection of RNA-protein interactions by Northwestern blotting and gel-shift assays further led to the isolation of two MIR-binding proteins having molecular weights (MW) of about 13-15 kDa and about 28-30 kDa. Comparison of MIR DNA sequences from normal and mutant embryos revealed a point mutation in the mutant DNA that resulted in the loss of functional (rescue) ability of the RNA, coupled with inability to bind the larger MW MIR-binding protein. Taken together, these results demonstrate that myofibrillogenesis and promotion of a normal cardiac muscle phenotype can be achieved through the interaction of MIR RNA with one or more MIR-binding proteins. (Emphasis added).

In view of the foregoing, applicants submit that claims 1 and 10 complies fully with 35 U.S.C. § 112, second paragraph. Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1 and 10.

The Examiner alleges on page 4, last paragraph of the Office Action, that it is unclear "what a MIR-binding protein is and there is no clear example of such a protein."

Applicants respectfully traverse.

Applicants teach that the MIR molecules specifically bind to proteins. See for example, page 14, lines 9-28, through to page 15, lines 1-8:

> The MIR molecules of the invention have been shown to specifically binds to MIR-binding proteins present in cells undergoing differentiation to a cardiac muscle phenotype. The discovery of the interaction of MIR with MIR-binding proteins holds great promise for inducing a cardiac muscle phenotype in a cell through use of MIR and its interacting proteins. Any protein can be used that specifically binds to MIR, leading to the induction of a cardiac muscle phenotype in a cell containing that protein. MIR-binding proteins can be isolated and identified by techniques known in the art, and further described herein. MIR-binding proteins in a cell or tissue can be separated for example in a first step by two-dimensional polyacrylamide gel (2D gel) electrophoresis, one of the most powerful methods to resolve complex protein mixtures. Although 2D gels are currently a widely used separation tool, reverse phase HPLC, capillary electrophoresis, isoelectric focusing and related hybrid techniques can also provide powerful means of resolving complex protein mixtures, and might also be used in the invention.
>
> In a second step, using a technique such as "Northwestern" blot analysis, the separated proteins can be tested for ability to bind to MIR, which is generally labeled with a detectable substance. Where a radioactive label is used as a detectable substance, a MIR-binding protein of the invention may be detected by autoradiography. The results of the autoradiography reveal those proteins separated within the 2D gel, i.e., "MIR-binding proteins," that display specific binding to the labeled MIR. Quantitation of the binding can be achieved by various optical methods. Confirmation of the specificity of the RNA-protein binding can be accomplished using a method such as a gel shift assay, in which binding of the radiolabeled MIR RNA to the protein is competitively challenged with increasing concentrations of unlabelled ("cold") MIR. Disappearance of a radioactive band representing a MIR:MIR-binding protein interaction in a dose-dependent manner is indicative of specific binding between the RNA and the protein.
>
> In preferred embodiments of the method, MIR-binding proteins can have MWs of ~11-13 kDa and ~28-30 kDa. As shown in examples herein, alkaline MIR-binding proteins of these sizes were identified by Northwestern blotting

using radiolabeled MIR as a probe of protein extracts from embryos undergoing cardiac morphogenesis. (Emphasis added).

As can be seen from the disclosure, an MIR-binding protein is a protein that binds to MIR. The MIR-binding proteins are characterized in the instant application as proteins that specifically bind to MIR and have been characterized by molecular weight of 11-13 kDa and 28-30 kDa. A detailed experimental protocol is disclosed in example 8, page 27, lines 13-27 through to page 28, lines 1-19. Results obtained are shown in Figures 6A and 6B. These MIR-binding proteins have not yet been named by applicants. Thus, applicants clearly teach what and MIR-binding protein is and have identified these proteins based on specific binding to the MIR molecules.

In view thereof, applicants submit that claims 2 and 4 fully comply with 35 U.S.C. § 112, second paragraph. Applicants respectfully request reconsideration and withdrawal of the rejection of claims 2 and 14.

"Claim 3 is indefinite because it is unclear what constitutes stringent hybridization conditions."

Applicants respectfully traverse.

Applicants define the varying stringent conditions for low, moderate or high stringency on page 5, lines 18-25. In order to make that which is implicit, explicit, applicants have amended claim 3 to recite that the complementary nucleic acid hybridizes under low, medium or high stringent hybridization conditions to the nucleotide sequence of at least one of SEQ ID NO: 2 and SEQ ID NO: 3.

Applicants respectfully request withdrawal and reconsideration of the 35 U.S.C. §112, second paragraph rejection as applied to claim 3.

"Claim 7 is drawn to a purified nucleic acid wherein a portion of the polynucleotide sequence shares sequence identity with a second sequence polynucleotide that encodes a RNA splicing factor."

Applicants respectfully traverse.

The sequence of the polynucleotide that encodes a RNA splicing factor has been disclosed by applicants as SEQ ID NO.: 7. The experimental data is shown in Example 16, page 32, lines 25-28, through to page 33, lines 1-13. Also see figures 8A and 8B.

Applicants have amended the specification and the claim 7, to include the reference to SEQ ID NO's.: 7 and 8.

Applicants respectfully request withdrawal and reconsideration of the 35 U.S.C. § 112, second paragraph rejection as applied to claim 7.

"Regarding claim 10, the phrase "about" renders the claim(s) indefinite because the claim(s) include elements not actually disclosed (those encompassed by "about"). Thereby rendering the scope of the claims unascertainable."

Applicants respectfully traverse.

Applicants have disclosed the entire sequence of the MIR molecule as identified by SEQ ID NO.: 5. Applicants further teach that the nucleotide sequence can be equal to or less than 166 nucleotides in length (see, for example, page 4, lines 8-10) or can be equal to or greater than 166 nucleotides in length (see, for example, page 4, lines 19-20). Therefore, the term "about" can include those nucleotides that are "about 167 nucleotides in length.

In order to expedite prosecution, however, applicants have amended claim 10 to recite "up to 620 nucleotides." Applicants submit that the term "about" is within the scope of the claimed invention.

Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. § 112, second paragraph rejection as applied to claim 10.

In view of the foregoing, claims 1-3, 7, 10 and 14 satisfy the requirements of 35 U.S.C. § 112, second paragraph. As such, these claims are allowable.

Claim Rejections Under 35 U.S.C. § 103.

Claims 1-3, 10, 11, and 13 are rejected under 35 U.S.C. § 102(b) as being anticipated by Lemanski *et al.*, (Lemanski *et al.*, *Biochem. Biophys. Res. Comm.* 229:974-981).

Applicants respectfully traverse.

Applicants disclose a full length sequence of a MIR-encoding nucleic acid molecule (SEQ ID NO.: 5), including sequences that are equal to or greater than 166 nucleotides in length. Furthermore, applicants disclose SEQ ID NO's.: 2 and 3 which hybridize under low, moderate or high stringent conditions to their complements. Furthermore, applicants teach the importance of the secondary structure of the MIR-encoding molecule and functions associated therewith. (See

above). Applicants teach the importance of a point mutation that destroys the functions of SEQ ID NO.: 5. See figure 5.

The sequences were not taught by Lemanski *et al.* and neither was their any disclosure or teaching as to hybridization conditions, the bioactivity of the MIR-encoding molecule related to specific nucleotide sequences. Although the sequence of clone #4 is embedded in the MIR-encoding molecule, (although the two sequences may not be exact), the sequence identified in Lemanski et al., would not posses the secondary structure of the full length MIR-encoding molecule as taught by Applicants.

With respect to claims 1, 7-9 and the RNA splicing factor SmN, the properties of clone #4 cDNA do not meet the limitations of claims 7-9. As discussed by applicants on page 33, lines 1-12, and shown in figure 8A, the SmN as represented by SEQ ID NO's.: 6 and 7 are not taught nor disclosed by Lemanski et al. SEQ ID NO's.: 6 and 7 do not match up to the clone #4 sequence shown in Figure 1 of Lemanski *et al.* In addition to the foregoing, the claims, as amended, precludes any teaching or suggestion by Lemanski *et al.*

It is respectfully submitted that for the foregoing reasons, claims 1-3, 10, 11, and 13 are patentable over the cited reference and satisfy the requirements of 35 U.S.C. §102. As such, these claims are allowable.

CONCLUSION

In view of the foregoing, reconsideration and withdrawal of all rejections and allowance of the application is respectfully solicited. If there are any remaining issues or the Examiner believes that a telephone conversation with the Applicants' representative would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at telephone number shown below. This response is accompanied by a petition for a three month retroactive extension of time and the required fee. The Commissioner for Patents and Trademarks is hereby authorized to charge the amount due for a three month retroactive extension of time and any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing, or during prosecution of this application to Deposit Account No. 50-0951.

Respectfully submitted,

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